

CAMPYLOBACTER IN CHICKEN CARCASSES AND SLAUGHTERHOUSES IN MALAYSIA

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Abstract. This study was conducted to determine the *Campylobacter* contamination rate of chicken carcasses and the processing lines of modern processing plants in Malaysia. Three hundred sixty samples were collected from 24 flocks of broiler chickens at 12 modern poultry processing plants in 6 states of Malaysia. Fresh fecal droppings were collected from crates in the arrival area. Neck skin samples were taken from processed chicken carcasses at 3 different processing stages: before inside-outside washing, after inside-outside washing and post chilling. Swab samples from the scalding tank, chilling tank and conveyer belt before chilling were also collected to determine contamination with *Campylobacter* in the slaughter house environment prior to slaughter. Isolation for *Campylobacter* was performed following ISO 10272-1:2006(E). The overall of contamination rate with *Campylobacter* at the 12 plants was 61.0% (220/360). Eighty point six percent of the samples from before the inside-outside wishing step were contaminated with *Campylobacter*, as were 62.5% of the samples after the inside washing and 38.9% after the post-chilling step. This study shows extensive contamination of chicken carcasses and slaughtering houses in Malaysia with *Campylobacter*.

Keywords: *Campylobacter*, chicken carcasse, slaughterhouse, Malaysia

INTRODUCTION

Campylobacter is a serious food-borne human pathogen and a common cause of gastroenteritis (Saleha *et al*, 1998). Its route

of transmission to humans is most commonly through ingestion of raw or undercooked poultry meat or milk, through pets and contaminated water (Ahmed, 2002). Most *Campylobacter* infections are caused by *Campylobacter jejuni* or *Campylobacter coli* (Anonymous, 2003). The Centers for Disease Control and Prevention (CDC) of the USA found *Campylobacter*, at an infectious dose as low as 500 organisms, is the most common bacterial cause of diarrheal illness in the United States, causing more illness than *Salmonella* and *Shigella* combined (Meer and Misner, 1998). One

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drop of fluid from a raw chicken can cause infection.

Malaysia is self-sufficient in providing its own needs for broiler chicken meat. Chicken is the most popular and economic source of animal protein for Malaysians; there are no religious restrictions against chicken consumption. About 30% of broiler chickens are channeled through modern processing plants in Malaysia, while the remaining 70% are sold as live or dressed birds in wet markets. There are health laws prohibiting chicken slaughter in wet markets in Malaysia, but enforcement of these law is challenging. Some wholesalers slaughter the birds in slaughterhouses and bring the dressed chicken to the marketplace (Loh *et al*, 2004).

Few studies of *Campylobacter* contamination of chickens have been carried out in Malaysia. Most studies were conducted to investigate the prevalence of *Campylobacter* at broiler chicken farms. Zeenathul (1994) reported a (97.1%) prevalence of *Campylobacter* spp among broiler chickens and 51.5% among village chickens. Another study conducted among broiler chicken farms in Malaysia found 72.6% of broiler chickens were colonized with *Campylobacter*; of these; *C. jejuni* was found in 73.2% and *C. coli* was found in 26.8% (Saleha, 2002).

One hundred thirty-seven children with *Campylobacter* diarrhea were reviewed in Malaysia. The predominant species was *C. jejuni*. The majority (95%) were <5 years old; 61% were 2-12 months old. About half the cases presented with fever and bloody diarrhea; vomiting was seen in 28% and abdominal pain in 8%. Moderate to severe diarrhea was observed in 48% of the children (Puthuchearry *et al*, 1994).

Acts, regulations, guidelines and

codes of practice related to food safety and wholesome quality are in place in Malaysia, but in spite of the efforts of regulatory agencies, there is still a problem of high microbial contamination with coliforms and pathogens in meat. A lot of effort had been made to improve the hygiene of slaughterhouses, but it is difficult to prevent contamination of carcasses with *C. jejuni* from their intestinal content (Berdntson *et al*, 1992; Ono and Yamamoto, 1999; Uyttendaele *et al*, 1999). In order to create an effective Hazard Analysis Critical Control Points (HACCP) program, it is important to know the efficacy of particular processing steps, considered as Critical Control Points (CCP) for microbial contamination. Since poultry is a major reservoir of *C. jejuni*, reducing contamination of poultry meat with this organism will decrease risk to consumers. Little information about contamination of meat with *Campylobacter* at processing plants is available from Malaysia. It is important to know the effect of processing steps (considered as CCPs) on microbial contamination. The objective of this study was to determine the contamination of chicken carcasses with *Campylobacter* during processing at modern poultry processing plants and the persistence of *Campylobacter* throughout the process.

MATERIALS AND METHODS

Sample size was estimated based on the total flock number slaughtered at all 12 processing plants from 6 States (800 flocks) from November 2008 to April 2009. The prevalence of *Campylobacter* in broiler chicken flocks on farms has been reported as 73% (Zeenathul, 1994). An assumed prevalence of 73%, with a 95% confidence interval and a 5% accepted error were used, so the total number of

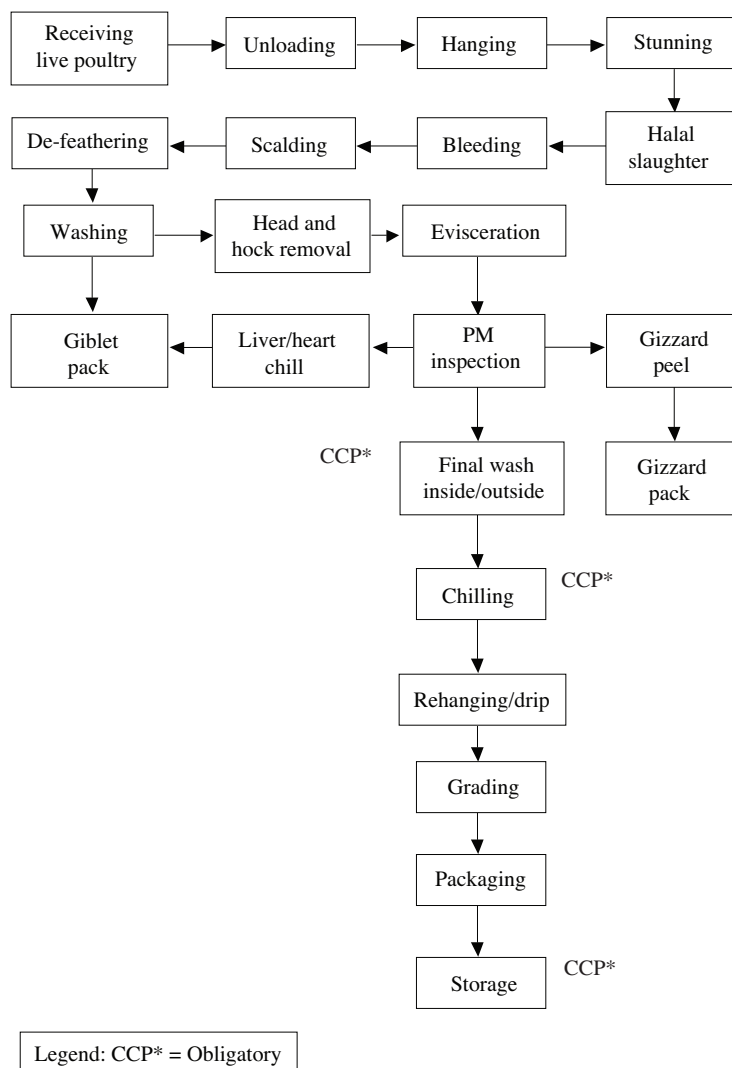


Fig 1—Standard poultry slaughter process flow chart in Malaysia.

calculated samples was 360 specimens. Samples were taken from 24 flocks (15 samples each) by swabbing the chicken carcasses and equipment for culture.

The studied processing plants practiced Good Manufacturing Practices (GMP) and HACCP during the slaughter and processing process. The slaughtering process is shown in Fig 1. Following stunning, Halal slaughter was carried out by a Muslim. The birds were left to bleed for 3-4 minutes then scalded at a tem-

perature of 56-62°C for 2 to 2.5 minutes. After scalding, the feathers were mechanically removed by a series of online plucking machines, where rubber finger-like projections remove the feathers from the carcasses. Evisceration is done mechanically; carcasses then pass through an automatic inside-outside bird washer. The carcasses are then immersed in a spin chiller for at least 20 minutes. The water is chlorinated at 20-40 ppm. The carcasses are then packed in plastic bags and placed in a chiller or freezer for delivery.

Slaughter per day varies between 15,000 to 140,000, at a rate of 1,500 to 6,000 chickens per hour. Samples were collected from 7 sites (Table 1) (4 sampling sites for each flock): upon arrival, before inside-outside washing, after inside-outside washing and post-chilling. Upon arrival, three fresh fecal dropping samples, 25 g each, were collected

randomly from transport crates. At the three other sampling sites, 25 g of neck skin each was taken from each of 3 carcasses from the same flock. Culture swab samples were taken from 3 sites (scalding tank, chilling tank and conveyer belt before chilling). Culture swab samples were collected from internal parts of equipment early morning before the slaughter process started. All samples were placed in a sterile bag and kept in an icebox until sent to the laboratory within 1-2 hours.

Table 1
Sampling scheme in processing plants.

Sampling site	Sample type	Number of samples
Equipment prior to slaughter:		
1. Scalding tank	Culture swab	1
2. Chilling tank	Culture swab	1
3. Conveyer belt before chilling	Culture swab	1
Samples from broilers		
4. Crates at arrival	Fresh fecal droppings	3
5. Before inside-outside washing	Processed chicken neck skin	3
6. After inside-outside washing	Processed chicken neck skin	3
7. Post-chilling	Processed chicken neck skin	3

Samples were stored at 3°C±2°C and analyzed within 24 hours.

A survey was conducted by questionnaire at all 12 processing plants involved in this study and the chickens' farm of origin asking about: Good Animal Husbandry Practices (GAHP), risk factors for contamination in the processing plants and data about the hygienic conditions of the abattoir.

Identification of *Campylobacter* spp was carried out as described by ISO 10272-1 (ISO, 2006). In brief, 25 g of neck skin/feces was added to 225 ml of enriched *Campylobacter* selective medium [Bolton broth (Oxoid, Cambridge, UK) with 5% lysed horse blood, polymyxin B (10,000IU/l), rifampicin (20 mg/l), trimethoprim (20mg/l) and cycloheximide (0.2 mg/ml)]. For swab samples, 1 swab sample was added to 90 ml of Bolton broth. It was then incubated under microaerobic conditions (7% O₂, 10% CO₂ and 83% N₂) at 37°C for 4 hours, and then at 42°C for 2 days. After culturing one loop of suspension was inoculated with a sterile loop onto the surface of a modified charcoal cefoperazone deoxycholate agar (mCCD agar) and Karmali agar (Oxoid) with sodium pyruvate (0.1 mg/l), cefoperazone (0.032 mg/l), vanco-

mycin (0.02 mg/l), amphotericin (0.01 mg). The samples were then incubated under microaerobic conditions for 44±4 hours at 42°C. Bacteria growth was checked daily. Suspected colonies were streaked onto Brucella agar (Oxoid,) containing 5% inactivated sheep blood and incubated under the above conditions for 24 hours. Typical colonies (round or irregular shape, white to clear with smooth edges) were harvested and the hanging drop motility test was done to see the cork screw motility typical of *Campylobacter*. Gram staining and biochemical tests (catalase and oxidase) were also performed to confirm the genus level. The Campy Latex agglutination test (Oxoid, Dry Spot *Campylobacter* Test) was conducted to confirm 4 species (*C. jejuni*, *C. coli*, *C. lari* and *C. upsaliensis*). The hippurate hydrolysis test was done to confirm *C. jejuni*. Isolates were stored by mixing in Bolton broth (Oxoid) with supplement and 20% (1vol/1vol) glycerine in cryovials at -80°C.

Laboratory and questionnaire data were entered into MS Excel 2003 database management software (Microsoft Office®, Washington, USA). Win Episcopo software (Win Episcopo®, Version 2.0, 1998) and STATA version 10 (STATA Corp,

Table 2
Campylobacter from different sites at poultry processing plants.

Sample site	Sample type	<i>Campylobacter</i> contamination level	95% CI
Equipment	Culture swabs (scalding tank, chilling tank and conveyer belt before chilling)	40.3% (29/72)	28.9-51.6
Crates on arrival	Fresh fecal droppings	83.3% (60/72)	74.4-91.9
Before inside-outside washing	Processed chicken neck skin	80.6% (58/72)	71.4-89.7
After inside-outside washing	Processed chicken neck skin	62.5% (45/72)	51.3-73.7
Post-chilling	Processed chicken neck skin	38.9% (28/72)	27.6-50.1

College Station, TX) were used for data management and analysis. The significance level and confidence intervals were set at 0.05 and 95%, respectively.

RESULTS

The overall rate of contamination with *Campylobacter* in modern processing plants was 61.0% (220/360). The prevalence of *C. jejuni* was 70.9% (156/220) with a 95% CI 64.9-77.0. The prevalence of *Campylobacter* prior to slaughter from the culture swab samples taken from scalding tanks, chilling tank and conveyer belt prior to slaughter was 40.3% (95% CI 28.9-51.6). The prevalence of *Campylobacter* for the crates on arrivals from live animals was 83.3% (95% CI 74.7-91.9).

The contamination rate of the processed chickens was significantly lower than on arrival ($p < 0.01$). The contamination rates with *Campylobacter* declined to 80.6% (95% CI 71.4-89.7) before the inside-outside washing step, 62.5% (95% CI 51.3-73.7) after inside washing and 38.9% (95% CI 27.6-50.1) post chilling (Table 2). These show a significant decrease in *Campylobacter* spp and *C. jejuni* contamination through the processing procedure (crate-

feces, before inside-outside washing, after inside-outside washing and post chilling) (Table 3).

The questionnaire results showed participants stated they were complying with GMP and HACCP guidelines. Ten thousand to 70,000 birds were slaughtered per day depending on the size and capacity of the plants and whether the plant had 1 or 2 shifts working. Nearly 70% of the chickens came from GAHP farms or farms certified with the Live-stock Farm Accreditation Scheme (SALT), the other 30% were supplied by farms in the process of implementing GAHP. The feed withdrawal period varied from 6 to 8 hours. The length of time needed to transport the birds from the farms to the slaughter house varied from 2 to 5 hours. The smaller plants had a single stage scalding tank while the bigger plants had a multistage scalding tank. The temperature of the scalding tank water ranged from 58°C to 62°C. Eleven plants used immersion chilling methods and 1 plant used an air chilling method. The antimicrobials used for the decontamination process in the chilling tank were sodium hypochlorite and chlorine dioxide. The chlorine concentration ranged from 20 to 40 ppm.

Table 3
Persistence of *Campylobacter* throughout the processing line.

Sampling site	Sample type	#Pos/Neg	OR	p-value	95% CI
Equipment	Culture swab	29/43	1	-	-
Crates on arrival	Fresh fecal droppings	60/12	7.41	0.000	3.10-17.71
Before inside-outside washing	Processed broiler carcass neck skin	58/14	6.14	0.000	2.68-14.03
After inside-outside washing	Processed broiler carcass neck skin	45/27	2.47	0.007	1.24-4.93
Post-chilling	Processed broiler carcass neck skin	28/44	0.94	0.865	0.48-1.84

DISCUSSION

This study showed *C. jejuni* enters poultry processing facilities through the birds, with subsequent contamination of equipment, processing lines and finally the poultry carcasses again. During processing, massive cross-contamination takes place. During scalding, the birds are washed as a result of turbulence in the water (Veerkamp, 1989). Each bird would transfer bacteria to the scalding tank. *Campylobacter* survive the scalding, are resistant to temperature increases and are attached to the chicken skin (Humphrey, 2004). This may be due to changes in the skin surface as a result of the scalding temperature, facilitating the attachment of bacteria (Jacobs-Retsma, 2000). Most modern processing plants have multistage scalding tanks. Izat *et al* (1988) found a significant reduction in *Campylobacter* counts (1.84 log) with multistage tanks and concluded this scalding technique was the most effective process for decreasing overall microbial levels on the surface of poultry carcasses. Scalding carcasses at 58°C to 62°C significantly reduced *Campylobacter* on chicken carcasses; however, de-feathering, evisceration and harvesting of giblets caused an increase in carcass contamination. This may be due to

viscera rupture during evisceration. This is further supported by the finding of high levels of *Campylobacter* contamination before inside-outside washing, just after the evisceration step.

The immersion chilling procedure has been identified as a CCP in a generic HACCP study of poultry contamination by all pathogens (Corry and Atabay, 2001). No CCP in poultry processing is capable of eliminating all pathogens. Carcasses examined after immersion chilling with a water temperature below 4°C and chlorine at 20 to 40 ppm had a significantly lower ($p < 0.01$) contamination level. The use of chlorinated water during carcass chilling reduced some *Campylobacter* but had only a limited effect on the magnitude of *Campylobacter* contamination.

The environment and equipment prior to slaughter were also contaminated. The finding of *Campylobacter* on and in equipment prior to slaughter suggests *Campylobacter* is still present after cleaning and disinfecting. This may be due to inadequate time between cleaning and disinfecting and the next slaughter. The short interval may be a factor in survival of *Campylobacter* due to the observation that the surfaces are still wet by that time with the next slaughter. In one study,

Campylobacter contamination of kitchen polypropylene surfaces significantly decreased after chlorine disinfection, but was still detectable (Cogan *et al*, 1999). This suggests campylobacter is likely to be present on slaughterhouse surfaces after routine cleaning and disinfection procedures.

Cleaning and disinfection is practiced routinely at modern poultry processing plants, but it fails to eliminate *Campylobacter*. One study found *C. jejuni* is able to survive overnight on food processing equipment surfaces after cleaning and disinfecting procedures, and these strains may contaminate carcasses during the slaughter process (Peyrat *et al*, 2008). Running an abattoir at high speed (up to 12,000 birds/hour) requires sophisticated technology. With increased line speed, service times used for hygiene become shorter, and the processing plants run in more than one shift. Bacterial loads and types in the air and water used for chilling are of great interest to better understand bacterial contamination in the processing environment (Fries and Graw, 1999).

Effective control measures in broiler chicken meat production are essential for *Campylobacter* prevention. Interventions to control *Campylobacter* should be carried out in three stages: pre-slaughter, during slaughter and post-slaughter. Alternative solutions need to be considered to improve efficacy. Interventions may include improved hygiene at the farm level, during slaughter and improved kitchen hygiene (Havelaar *et al*, 2006).

Interventions before slaughter include preventing flocks from being colonized with *Campylobacter* and interventions to reduce the concentration of *Campylobacter* after colonization. Since horizontal transmission is the most common route of infection, intervention measures that

tackle horizontal transmission should be considered. The most important interventions before slaughter are biosecurity measures in and around farms, insect control (fly screens) and improved techniques for thinning flocks. Bio-security measures at the farm level must be maintained, even though these measures can only provide a preliminary barrier.

Interventions during slaughter include hygienic measures and interventions to reduce fecal contamination (by following GMP). Contamination during scalding can be minimized by using counter-current flow, multistage scalding tanks, the addition of as much fresh water as possible and the use of approved chemicals. Improving the washing system used during processing called an inside-outside bird washer, may help reduce the number of *Campylobacter* spp on carcasses (Oyarzabal, 2005). However, the reduction may not be significant, or be achieved consistently. The effectiveness of an inside-outside final wash to reduce *Campylobacter* depends greatly on water volume, pressure and level of chlorine in the water. The immersion chilling procedure has been identified as a CCP in a generic HACCP study of poultry contamination by all pathogens. A decrease in *Campylobacter* counts associated with chilling operations has also been reported previously, indicating the possibility of achieving reductions of *Campylobacter* up to 2 log₁₀ colony forming unit on carcasses. Studies evaluating the performance and effectiveness of poultry washers and sanitizing measures within processing plants are limited, so little is known about the numerous factors affecting the overall efficiency of a carcass washing system, including the number and types of washers (Anand *et al*, 1989; Dickens and Cox, 1992; Bautista *et al*, 1997).

Post-slaughter measures for decon-

tamination include interventions aimed at reducing bacterial concentration on carcasses with chemical treatment (acidified sodium chlorite, chlorine, chlorine dioxide, trisodium phosphate, cetylpyridinium chloride, ozone and per-oxy acids) and physical treatment (freezing, crust freezing, steam-ultrasound, steam/hot water, forced air chilling, heat treatment and irradiation) (EFSA, 2008).

Campylobacter contamination is widespread in live chickens before slaughter, in the processing plant environment and on processed chicken carcasses. *Campylobacter* concentration is substantially decreased when the slaughter occurs in modern processing plants. Malaysian modern/commercial processing slaughter plants are capable of having lower contamination rates on broiler chicken carcasses during processing.

Reducing the number of *Campylobacter* on poultry carcasses can be achieved by reducing fecal contamination during slaughter and further processing, and by using appropriate physical (eg, freezing) or chemical decontamination techniques, where permitted. Implementation of advanced technical steps such as air chilling, counter flow and multistage scalding tanks (Cason *et al*, 1999, 2000) and further development of steam scalding; mechanical removal of feces from the rectum (Heemskerck, 2005) may be helpful in reducing microbial contamination.

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